Am. J. Hum. Genet. 73:1282–1292, 2003

MDR1 Ala893 Polymorphism Is Associated with Inflammatory Bowel Disease

Steven R. Brant,[1,2] Carolien I. M. Panhuysen,[3] Dan Nicolae,[4] Deepthi M. Reddy,[1] Denise K. Bonen,[5] Reda Karaliukas,[5] Leilei Zhang,[6] Eric Swanson,[5] Lisa W. Datta,[1] Thomas Moran,[5] Geoffrey Ravenhill,[1] Richard H. Duerr,[6,7] Jean-Paul Achkar,[8] Amir S. Karban,[1] and Judy H. Cho[5]

[1]The Harvey M. and Lyn P. Meyerhoff Inflammatory Bowel Disease Center, Department of Medicine, Johns Hopkins University School of Medicine, and [2]Department of Genetic Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore; [3]Boston University School of Medicine, Boston; Departments of [4]Statistics and [5]Medicine, The Martin Boyer Laboratories, Section of Gastroenterology, University of Chicago Hospitals, Chicago; [6]Division of Gastroenterology, Hepatology and Nutrition, Department of Medicine, School of Medicine, and [7]Department of Human Genetics, Graduate School of Public Health, University of Pittsburgh, Pittsburgh; and [8]Department of Gastroenterology, The Cleveland Clinic Foundation, Cleveland

Crohn disease (CD) and ulcerative colitis (UC) are overlapping chronic inflammatory bowel diseases (IBDs). Suggestive evidence for linkage at chromosome 7q has been reported for both CD and UC. Contained within this region is the gene for *MDR1* (multidrug resistance), a membrane transport protein for which human polymorphisms have been reported in Ala893Ser/Thr and C3435T that alter pharmacokinetic profiles for a variety of drugs. Because *mdr1* knockout mice spontaneously develop colitis, exonic regions were resequenced and tested for IBD association in a large, multicenter North American cohort. Two missense mutations, Asn21Asp and Ala893Ser/Thr, as well as the expression-associated polymorphism C3435T, described elsewhere, were genotyped in the entire cohort. Significant association of Ala893 with IBD was observed by both case-control analysis ($P = .002$) and the pedigree disequilibrium test (PDT [$P = .00020$–$.00030$]) but not for the Asn21Asp or C3435T polymorphisms. Significant association by PDT was observed within the subset with CD ($P = .0014$–$.00090$), with similar, nonsignificant trends in a smaller subset with UC. The Ala893Ser/Thr variant is triallelic, and the associated, common allele is Ala893, with undertransmission of the 893Ser (common) and the 893Thr (rare) variants. The Ala893 variant has decreased activity compared with the 893Ser variant; therefore, the association with human IBD is consistent with the murine model of *mdr1* deficiency. Taken together, these data support the association of the common Ala893 polymorphism with IBD specifically and, more broadly, provides additional support for its contribution to interindividual pharmacogenetic variation.

Introduction

The idiopathic inflammatory bowel diseases (IBDs) comprise Crohn disease (CD [MIM 266600]) and ulcerative colitis (UC [MIM 191390]), with both disorders involving chronic, intermittent inflammation that responds to anti-inflammatory agents such as corticosteroids and immunosuppressive therapies (Podolsky 2002). The distinction between UC and CD is based largely on the distribution of inflammation; the inflammation of UC invariably involves the rectum and extends, to a variable extent, proximally throughout the colon in a symmetric, continuous manner and affects only the innermost layers of the colonic wall. In contrast, the inflammation of CD can affect any portion of the gastrointestinal tract, with involvement of the terminal ileum of the small intestine being the most commonly affected portion. Inflammation in CD is often patchy, asymmetric, and discontinuous in nature (where intensely involved regions are separated by uninvolved, "skip" regions) and can involve all layers of the bowel wall. Aggressive transmural involvement in CD accounts for complications involving intestinal perforation, fistulous connections between intestine and/or adjacent tissues, and intestinal obstructions from strictures.

That genetic factors contribute to CD and UC pathogenesis is indicated by the consistent findings between studies that MZ twin concordance is significantly higher than DZ twin concordance (Tysk et al. 1988; Thompson et al. 1996; Subhani 1998). For CD, MZ twin concordance rates are 42%–58%, whereas, for UC, estimated

Received August 20, 2003; accepted for publication September 12, 2003; electronically published November 7, 2003.

Address for correspondence and reprints: Dr. Steven R. Brant, Johns Hopkins University School of Medicine, 1503 East Jefferson Street, Room B136, Baltimore, MD 21231. E-mail: sbrant@jhmi.edu; or Dr. Judy H. Cho, University of Chicago Hospitals, 5841 South Maryland Avenue, Room G705, Chicago, IL 60637. E-mail: jcho@medicine.bsd.uchicago.edu

0002–9297/2003/7306-0008$15.00

concordance has a range of 6%–17%. The relative risk to first-degree relatives of a proband with IBD of developing the same disease is 4–10-fold (Orholm et al. 1991; Peeters et al. 1996). Furthermore, among families having multiple members with IBD, 25% are mixed—that is, they have members with CD as well as members with UC (Binder 1998). Taken together, these data are consistent with a model in which some risk alleles and/or susceptibility genes will be common to both disorders, whereas other susceptibility genes will be unique to either CD or UC.

A number of genomewide searches for IBD susceptibility loci have been published, and, as with other multigenic disorders, initially reported linkages have been variably observed in subsequent studies (Hugot et al. 1996; Satsangi et al. 1996; Cho et al. 1998; Hampe et al. 1999; Ma et al. 1999; Duerr et al. 2000; Rioux et al. 2000; Williams et al. 2002; Paavola-Sakki et al. 2003). Furthermore, some loci have been linked primarily with CD or UC, whereas, for other loci, linkage has been observed in both IBD subsets. The best example of a CD-specific locus is *IBD1* in the pericentromeric region of chromosome 16 (Hugot et al. 1996), which is now known to be partially due to the presence of three major coding region polymorphisms in NOD2/CARD15 (Hugot et al. 2001; Ogura et al. 2001). These mutations decrease innate immune responsiveness (Bonen et al. 2003) to muramyl dipeptide (Girardin et al. 2003; Inohara et al. 2003), a minimally active component of peptidoglycan found in the cell walls of gram-positive and -negative bacteria.

Suggestive evidence for linkage at chromosome 7q near D7S669 was initially reported in a European cohort consisting of 186 affected sibling pairs from 160 families (Satsangi et al. 1996). Subsequent studies demonstrated nominal evidence for linkage in this region, including our genomewide screen (Cho et al. 1998), which demonstrated nominal evidence for linkage in all families with IBD and, in particular, among mixed families (containing both CD- and UC-affected members) (maximal multipoint LOD = 1.18).

Contained within this linkage region on chromosome 7q is the multidrug resistance 1 gene (*MDR1* or ATP-binding cassette subfamily B member 1, *ABCB1* [MIM 171050]). The *MDR1* gene product, P-glycoprotein-1 (PGP), was initially observed as a transport protein increased in tumors developing resistance to chemotherapeutic agents, resulting from PGP-mediated efflux of drug from tumor cells (Choi et al. 1988). Others proposed that PGP functions as a "flippase"—as opposed to an efflux pump—moving amphipathic substrates from the inner leaflet to the outer leaflet of the cell membrane, with the ability to move these substrates prior to their penetration into the cytoplasm (Higgins and Gottesman 1992; van Helvoort et al. 1996). PGP is a 12-transmembrane–spanning ATP-binding transporter of lipophilic molecules (including some chemotherapeutic agents) across plasma membranes (Ueda et al. 1987; Jones and George 1998). Variation in *MDR1* expression and/or genotypes has been related to altered pharmacokinetics of a wide range of drugs, including corticosteroids (Schinkel et al. 1995; Lown et al. 1997; Farrell et al. 2000), immunosuppressives (Lown et al. 1997; Yamauchi et al. 2002), digoxin (Hoffmeyer et al. 2000; Kim et al. 2001; Johne et al. 2002; Verstuyft et al. 2003), antihistamines (fexofenadine) (Kim et al. 2001), and anticonvulsants (Siddiqui et al. 2003). Because *MDR1* is broadly expressed, alterations in *MDR1* expression and activity may result in altered intestinal absorption (Lown et al. 1997; Sparreboom et al. 1997; Hoffmeyer et al. 2000; Kim et al. 2001), leukocyte transport (Hitzl et al. 2001; Calado et al. 2002), blood-brain barrier function (Schinkel et al. 1995, 1996; Furuno et al. 2002; Yamauchi et al. 2002; Siddiqui et al. 2003), and urinary excretion (Schinkel et al. 1995).

It is significant that, although developmentally normal, *mdr1*-deficient mice spontaneously develop colitis (Schinkel et al. 1997). This suggests that functional polymorphisms within *mdr1,* which result in a loss of or decrease in PGP activity, might increase susceptibility to IBD (Panwala et al. 1998). Among germline polymorphisms within *MDR1,* there has been some controversy regarding the functional significance of a missense polymorphism contained within exon 21 (G2677T/C-Ala893Ser/Thr) and of a wobble polymorphism contained within exon 26 (C3435T-Ile1145Ile). Various reports have suggested that one or the other are associated with altered transporter and/or gene-expression activity (Hoffmeyer et al. 2000; Hitzl et al. 2001; Kim et al. 2001; Calado et al. 2002; Johne et al. 2002; Siegmund et al. 2002; Yamauchi et al. 2002; Verstuyft et al. 2003). Recently, in a case-control study, the 3435T variant was found to be significantly associated with UC but not with CD (Schwab et al. 2003). Of note, however, the Ala893Ser/Thr polymorphism was not examined in that study. In this article, we examine the Asn21Asp variant, as well as functionally associated polymorphisms on Ala893Ser/Thr and C3435T. We observe significant evidence for CD association for the Ala893 variant, which has been associated with decreased activity relative to the 893Ser variant (Kim et al. 2001). Furthermore, we observe no evidence for UC or CD association for the 3435T variant.

Material and Methods

Family Ascertainment

In all cases, informed consent was obtained for molecular genetics studies approved by the institutional review boards at Johns Hopkins University, University of

Pittsburgh, and University of Chicago Hospitals. Diagnoses were confirmed by review of primary medical records, including radiologic, endoscopic, and pathology reports. Table 1 summarizes the families genotyped by diagnosis, ethnicity, and family structure. At least one completely informative trio was available for each of the families included in the study. A total of 329 families with IBD was genotyped, with 35% of families of Ashkenazi Jewish ancestry and 4 families with Jewish ethnicity status unknown. In all, 1,188 subjects were genotyped, including 558 subjects with IBD. For the case-control analysis, 392 non-Jewish and 219 white Ashkenazi Jewish healthy control individuals of European ancestry were genotyped. These control individuals were ascertained from Johns Hopkins University, the University of Chicago, and (for additional Ashkenazi Jewish control individuals) a population-based cohort study. (The New York Cancer Project control subjects are healthy individuals, randomly ascertained for longitudinal follow up for future development of cancer.)

Table 1

Structure of IBD Pedigrees Genotyped

	No. with Affection Status		
Pedigree Structure	IBD	CD	UC
Families[a]	329	264	84
Affected individuals[b]	558	409	119
Trios	444	353	88
Simple trio pedigrees[c]:	223	189	71
Non-Jewish	146	121	47
Jewish	73	64	24
Sibling pair pedigrees[d]:	86	62	10
Non-Jewish	51	38	5
Jewish	35	24	5
Extended pedigrees[e]:	20	13	3
Non-Jewish	14	9	2
Jewish	6	4	1

[a] Twenty-two families with IBD had offspring with both UC and CD; the offspring with UC and CD were also analyzed separately with the other pedigrees that had offspring with only UC and CD, respectively. Three families had offspring with only indeterminate colitis and were included in the IBD analyses but not in the CD or UC analyses.

[b] The number of affected individuals listed for families with offspring with CD include only pedigree members with CD; the number of affected individuals listed for families with offspring with UC include only pedigree members with UC. Affected parents are also included.

[c] Includes three simple trio pedigrees with indeterminate colitis. Because of the presence of multiple mixed sibling pair and mixed extended pedigrees, the total number of simple trio pedigrees with IBD is less than the total number of simple trio pedigrees with CD and UC. Four trio pedigrees with CD were unknown for Jewish ethnicity.

[d] The CD analyses include 4 pedigrees with CD with 3 affected siblings, 2 pedigrees with CD with 4 affected siblings, and 18 mixed pedigrees (16 having one sibling pair with CD and one sibling pair with UC, 1 mixed pedigree composed of two siblings with CD and two siblings with UC, and 1 mixed pedigree composed of two siblings with CD and one sibling with UC).

[e] Pedigrees with more than one trio and containing one or more affected cousin pairs, avuncular pairs, or DNA samples from three generations with parent-child pairs.

SNP Genotyping

Table 2 summarizes the rs designation, amino acid and nucleotide changes, and exon locations for the three typed SNPs. The Ala893Ser/Thr polymorphism was genotyped by PCR sequencing (forward primer: 5′-CTGATAAAATAATGAATATAGTCTC-3′; reverse primer: 5′-TAGAGCATAGTAAGCAGTAGG-3′) by use of dye-terminator chemistry (ABI3700 sequencer) and pyrosequencing (PSQ HS96A system) (forward primer: 5′Biotin-TEG-GAGCATAGTAAGCAGTAGGGA-3′; reverse primer: 5′-TACCCATCATTGCAATAGC-3′; pyrosequencing primer: 5′-GATAAGAAAGAACTAGAAGG-3′). The Asn21Asp (forward primer: 5′-ATGGAGGAGCAAAGAAGAAGAACTT-3′; reverse primer: 5′-CGCAACTATGTAAACTATGAAAATGAAAC-3′; VIC [WT probe]: 5′-AACTGAACAATAAAAGG-3′; FAM [rare probe]: 5′-CTGAACGATAAAAGG-3′) and C3435T polymorphisms (forward primer: 5′-AACAGCCGGGTGGTGTCA-3′; reverse primer: 5′-ATGTATGTTGGCCTCCTTTGCT-3′; VIC [WT probe]: 5′-CTCACGATCTCTTC-3′; FAM [rare probe]: 5′-CCTCACAATCTCTT-3′) were genotyped using *Taq*man amplification and were detected using the ABI Prism 7700 Sequence Detection System.

Analysis

The data were checked for Mendelian inconsistencies, and the files were prepared using the Sib-Pair program (Duffy 1997). The same program was used to check for deviation from Hardy-Weinberg equilibrium. For the case-control analysis, one random affected individual of either known white European or Ashkenazi Jewish ancestry was selected from each family. A similar proportion of ethnically matched control subjects was genotyped for comparisons. The differences in allele and genotype frequency between two independent samples (subjects vs. control individuals and Jewish control individuals vs. white non-Jewish control individuals) were tested using χ^2 statistics for two-way tables. The combined white non-Jewish and Jewish test statistic was calculated as the sum of the individual χ^2 statistics. The P values were obtained using Monte Carlo simulation.

Association analyses were performed using a pedigree-based transmission/disequilibrium test (PDT) (Martin et al. 2000). This program allows for inclusion of triads, as well as extended families, in the analysis, while adjusting for their nonindependence. The PDT is a powerful analytical method that utilizes genetic data from related nuclear families and discordant sibships within extended pedigrees. It has two global scores: the "sumPDT," which

Table 2

Allele Frequencies for Three MDR1 Polymorphisms in Control Individuals and Patients with IBD, Stratified by Jewish Ethnicity

rs Designation and Amino Acid	Nucleotide	Exon	White Non-Jewish Subjects with IBD (%)	White Non-Jewish Control Individuals (%)	Jewish Subjects with IBD (%)	Jewish Control Individuals (%)
Pending:						
Asn21	A61	2	91.3	89.1	96.4	96.3
21Asp	61G	2	8.7	10.9	3.6	3.7
rs2032582:						
Ala893	G2677	21	60.2	52.4	62.7	60.5
893Ser	2677T	21	39.3	45.0	36.8	36.5
893Thr	2677A	21	.5	2.6	.4	3.0
rs1045642:						
Synonymous	C3435	26	50.0	45.4	57.7	64.3
Synonymous	3435T	26	50.0	54.6	42.3	35.7

summarizes the level of significance from all families, and the "avePDT," for which the contribution of large families to the end result does not exceed that of the small families. Distortion of transmission from parents to offspring is assessed by an observed/expected χ^2 test. The analysis was performed for the general phenotype of IBD and then separately for CD and UC. The r^2 linkage disequilibrium value for SNP pairs was estimated using the method of Weir (1996). Transmission/disequilibrium test (TDT) and haplotype analysis were performed using Genehunter, version 2.1 (Kruglyak et al. 1996; Kong and Cox 1997; Markianos et al. 2001).

Results

Resequencing of MDR1 *Exons*

We determined the sequence of all 27 *MDR1*-coding exons in DNA samples from 18 independent patients with IBD. These patients were all probands from the 18 multiply IBD-affected pedigrees who demonstrated the strongest evidence for linkage on chromosome 7q21.1 (at the *MDR1* map location between markers D7S2204 and D7S820) in our IBD genomewide screen reported elsewhere (Cho et al. 1998). We identified four exonic SNPs: in exon 2 (A61G-Asn21Asp), exon 12 (C1236T), exon 21 (G2677T/A-Ala893Ser/Thr), and exon 26 (C3435T) (table 2). We also identified two SNPs in flanking introns 6 and 17. Of note is that Ala893Ser/Thr is a triallelic amino acid polymorphism that results either in the common alanine or serine variants or in the least common threonine residue. The Ala893 and 893Thr exon 21 allelic variants were found only on the background of the more common Asn21 exon 2 variant and not on the 21Asp variant.

Because the Ala893Ser/Thr and C3435T polymorphisms have been associated with altered *MDR1* function, and because the Asn21Asp polymorphism represents a nonconserved amino acid variation that could potentially alter function, we typed these variants in a large cohort of patients with IBD to test for disease association.

Case-Control Analysis Demonstrates Association of Ala893 to IBD

Table 2 lists the allele frequencies for the typed markers in white non-Jewish and Jewish cohorts. For the Asn21Asp variant, no evidence for IBD association was observed ($P = .36$). For the C3435T polymorphism, marked differences in the C3435 allele frequencies were observed between white non-Jewish (45.4%) and Jewish (64.3%) control individuals ($P = 2.2 \times 10^{-16}$). No evidence for IBD association was observed for this polymorphism in either cohort, with an increase of the C3435 allele in the non-Jewish cohort and a decrease of the C3435 allele in the Jewish cohort.

For the Ala893Ser/Thr variant, the Ala893 variant was modestly increased in Jewish control individuals, as compared with non-Jewish control individuals, with a respective decrease in the 893Ser variant and a similar presence of the relatively rare 893Thr variant. The global difference in allele frequencies between Jewish and non-Jewish control individuals for Ala893Ser/Thr is modestly significant ($P = .015$). It is important that the Ala893 allele was increased in both white non-Jewish and Jewish subjects with IBD, as compared with their ethnically matched control individuals. Table 3 lists the allele and genotype counts for the Ala893Ser/Thr variants in white non-Jewish and Jewish cohorts. While counting alleles, we observed significant association for Ala893Ser/Thr for white non-Jewish subjects with IBD ($P = .003$) and a modest trend for association among Jewish subjects with IBD ($P = .09$). The combined significance in both cohorts (calculated as the sum of the individual statistics) was $P = .002$. There were no significant differences between CD Ala893 and UC allele frequencies in white non-Jewish (60.9% vs. 61.7%, respectively) and Jewish (63.8% vs. 64.5%, respectively) cohorts. However, because the number of UC cases is small, we would have

Table 3

Allele and Genotype Counts for Control Individuals and Patients with IBD, Stratified by Ethnicity

Allele or Genotype	No. of White Non-Jewish Subjects with IBD	No. of White Non-Jewish Control Individuals	No. of Jewish Subjects with IBD	No. of Jewish Control Individuals
Allele:				
Ala893	254	411	143	265
893Ser	166	353	84	160
893Thr	2	20	1	13
Genotype:				
Ala893-Ala893	76	108	41	85
Ala893-893Ser	101	183	60	89
893Ser-893Ser	32	81	12	32
Ala893-893Thr	1	12	1	6
893Ser-893Thr	1	8	0	7

Note.—Differences between subjects and control individuals in each cohort were tested using the χ^2 statistics for two-way tables, with significance estimates obtained by Monte Carlo simulation. For alleles and genotypes, significant association was observed for Ala893Ser/Thr for white non-Jewish subjects with IBD ($P = .003$ and $P = .015$, respectively), and a modest trend for association was observed among Jewish subjects with IBD ($P = .09$ and $P = .072$, respectively). The combined analysis of the two ethnic groups (calculated as the sum of the individual cohort statistics) was $P = .002$ for alleles and was $P = .008$ for genotypes.

limited power to identify modest differences. More generally, the marked allele frequency differences in *MDR1* variants in various populations highlight the difficulty of appropriately controlling for cryptic population substructure in the case-control–study design. In addition, haplotype analysis might provide further insight into the relationship between the *MDR1* variants under investigation. The following sections summarize the results from family-based association studies.

Significant Association of IBD and CD with the Ala893Ser/Thr Polymorphism by Family-Based Association Studies

Because the TDT is a test of both association and linkage, in a cohort such as ours with a large number of families with more than one affected member, the significance values obtained by TDT do not reflect disease association solely. A conservative correction would be to randomly select one IBD-affected individual from each family. Using this approach, we observed significant association of Ala893 to IBD in all families with IBD (165 transmissions, 119 nontransmissions; $\chi^2 = 7.45$; $P = .0063$) and in white non-Jewish families (112 transmissions, 79 nontransmissions; $\chi^2 = 5.7$; $P = .017$) but not in Jewish families (53 transmissions, 40 nontransmissions; $\chi^2 = 1.82$; $P = .18$). These results nicely mirror the cohort differences observed by case-control study (table 2), where a greater Ala893 allele-frequency difference was observed for white non-Jewish subjects with IBD (60.2% vs. 52.4% in subjects and control individuals, respectively) compared with Jewish IBD (62.7% vs. 60.5%). Also similar to the case-control findings, we observed marked undertransmission of the 893Thr allele in both Jewish (zero transmissions, six nontransmissions) and white non-Jewish (two transmissions, seven nontransmissions) when randomly selecting one IBD-affected subject per family.

Table 4

Global *P* Values by PDT Analysis of Exonic SNPs

SNP	Global *P* for PDT Analysis for CD Sum	CD Average	UC Sum	UC Average	IBD Sum	IBD Average
Asn21Asp	.24	.15	.26	.56	.8	.39
Ala893Ser/Thr	.0014	.00090	.29	.16	.00030	.00020
C3435T	.42	.31	.93	.60	.63	.35

A more powerful test of association in a cohort such as ours is the PDT (Martin et al. 2000), which utilizes genetic data from related nuclear families and discordant sibships within extended pedigrees. Table 4 demonstrates the results by PDT for the Asn21Asp, Ala893Ser/Thr, and C3435T polymorphisms among patients with CD, UC, and IBD. These PDT values represent transmission disequilibrium independent from linkage. For the entire cohort with IBD, significant evidence for association was observed for the Ala893Ser/Thr polymorphism ($P = .00020$–$.00030$), and no evidence was observed for the Asn21Asp or C3435T polymorphisms. Separate evaluation by CD and UC phenotypes showed no evidence of a CD association for the Asn21Asp or the C3435T polymorphisms. In contrast, significant CD association was observed by PDT for the Ala893Ser/Thr polymorphism ($P = .00090$–$.0014$). Similar but nonsignificant trends are observed in UC for the Ala893Ser/Thr polymorphism ($P = .16$–$.29$). Because the number of subjects with UC is significantly smaller than the num-

Table 5

PDT Analysis of the Tri-Allelic Exon 21 SNP

		P for PDT Analysis for					
		CD		UC		IBD	
Allele	Transmission	Sum	Average	Sum	Average	Sum	Average
Ala893	Overtransmitted	.0072	.0040	.31	.15	.0021	.0010
893Ser	Undertransmitted	.036	.028	.43	.23	.016	.0093
893Thr	Undertransmitted	.0046	.0046	.16	.16	.0025	.0032
Global score		.0014	.00090	.29	.16	.00030	.00020

ber of subjects with CD in this cohort, these trends do not support a significant association of the Ala893Ser/Thr polymorphism with UC. In the cohort with UC, in contrast to a prior case-control study (Schwab et al. 2003), we observed no evidence for association with the 3435T polymorphism, nor was evidence observed for the Asn21Asp.

For diallelic SNPs, the global PDT significance estimates equal that for each of the variants individually. In contrast, for triallelic SNPs, the global PDT estimates comprise unique contributions from each of the three variants. Subanalyses of each of the three (Ala893, 893Ser, and 893Thr) alleles showed that the overall Ala893Ser/Thr associations for IBD and CD result from significant overtransmissions of the Ala893 variant and undertransmissions of the 893Ser and 893Thr variants (table 5).

Of note is that the least common variant, 893Thr, was associated with marked undertransmission (counting all transmissions: 2 transmissions, 18 nontransmissions; and by PDT: $P = .0025$–$.0032$) to IBD-affected individuals. This marked undertransmission of a rare variant provides additional support for association of the Ala893Ser/Thr polymorphism and IBD. Therefore, for the triallelic Ala893Ser/Thr polymorphism, the global *P* value by PDT is lower ($P = .00020$–$.00030$) than that for any allele by itself.

Haplotype TDT Analysis Demonstrates No Additional Evidence for Association beyond That Observed for the Ala893Ser/Thr Polymorphism Alone

Table 6 lists the r^2 values for each pair of typed SNP. Because of the significant linkage disequilibrium between Ala893Ser/Thr and C3435T, we performed haplotype TDT (table 7) to define their relationships in this cohort.

Table 6

Linkage Disequilibrium Values

SNP Pair	r^2
Asn21Asp and Ala893Ser/Thr	.10
Ala893Ser/Thr and C3435T	.52
Asn21Asp and C3435T	.09

Specifically, because the 3435T variant had been reported elsewhere to be associated with UC by case-control analysis, we sought to define the relationship between Ala893Ser/Thr and C3435T variants, with respect to the evidence for PDT association in IBD.

Two-locus–haplotype analysis of the Asn21Asp-Ala893Ser/Thr haplotype demonstrates that the less common 21Asp variant occurs solely on the Ser893 background, which is unassociated with disease. In contrast, the Asn21-Ala893 variant represents the associated haplotype (203 transmissions, 128 nontransmissions). Preferential transmission of the Ala893Ser/Thr-C3435T haplotypes is observed for the Ala893 variant occurring with either the C or T allele at C3435T (table 7). Therefore, whereas significant evidence for IBD association is observed for the Ala893 allele by itself, no association is observed for IBD for the C3435T polymorphism by single-point or haplotype TDT.

Discussion

Given the unique, close apposition of the distal small and large intestine to high concentrations of potentially noxious bacteria, a unifying model for genes that increase susceptibility to IBD is that they fundamentally affect host-environment interactions. Just as NOD2/CARD15 risk alleles result in decreased responsiveness to bacterial cell wall products (Ogura et al. 2001; Bonen et al. 2003; Chamaillard et al. 2003), the Ala893 variant in *MDR1* has been associated with relatively decreased transporter function (Kim et al. 2001), including potentially noxious xenobiotics (Schinkel 1997). In the *mdr1* knockout model with spontaneously developing colitis (Panwala et al. 1998), as with most IBD models, the inflammation required the presence of intraluminal bacteria and could be prevented (or treated) with oral antibiotics. Furthermore, bone marrow–chimera studies showed that *mdr1* deficiency in radioresistant (e.g., intestinal epithelium) cells—not *mdr1* deficiency in hematopoietic cells—was the critical contributing factor (Panwala et al. 1998).

By sequencing 18 individuals throughout *MDR1* exons and flanking introns, we would have >95% power to identify SNPs of ⩾5% allele frequency (Kruglyak and

Table 7

TDT for Two-Locus Haplotypes for All Patients with IBD

Haplotype	No. of Haplotypes	
	Transmitted	Untransmitted
Asn21Asp–Ala893Ser/Thr:		
Asn21–Thr893	2	17
Asn21–Ala893	203	128
Asn21–Ser893	131	188
21Asp–Ser893	48	51
Ala893Ser/Thr–C3435T:		
893Thr–C3435	0	14
893Thr–3435T	2	2
Ala893–C3435	174	133
Ala893–3435T	69	51
893Ser–C3435	14	31
893Ser–3435T	130	158

Note.—Haplotypes were constructed using Genehunter, version 2.1; all affected individuals were included.

Nickerson 2001). We observe significant evidence for association of both CD and IBD to the Ala893 allele by both case-control analysis and PDT. Although the trend for association of Ala893 in the UC cohort alone is similar to that for CD, it is likely that, because of the significantly smaller numbers of subjects with UC, the association was not significant. By both case-control analysis (table 3) and TDT testing for one affected individual per family, we observe a greater association of Ala893 in white non-Jewish, compared with Jewish, cohorts. This reflects both the higher number of white non-Jewish families tested and the relatively modest allele frequency difference for Ala893 in Jewish subjects with IBD (62.7%) compared with control subjects (60.5%). Furthermore, the Jewish control Ala893 frequency (60.5%) is essentially equal to that of the white non-Jewish subjects with IBD (60.2%) (table 3). By comparison, in 461 German control subjects, Ala893 was observed at a 56.4% allele frequency (Cascorbi et al. 2001). Given the higher IBD prevalence in Jews, it is not unexpected that a common IBD risk allele would be higher in Jewish, compared with white non-Jewish subjects with IBD and control individuals.

These very subtle allele frequency differences between cohorts highlight the limitations of case-control analyses and the importance of our present family-based association findings (table 4). Ala893Ser/Thr is triallelic (table 5). Each of the three alleles showed independent distortion in transmission frequencies from that expected. It is notable that, because the rare 893Thr variant is associated with a marked undertransmission to probands with IBD (2 transmissions, 18 nontransmissions), the overall positive (Ala893) and negative (893Thr, 893Ser) associations of codon 893 to CD ($P = .0014$–$.00090$) and IBD ($P = .00020$–$.00030$) are more significant than for any single allele by itself.

We observed marked differences in C3435 allele frequency between white non-Jewish (45.4%) and Jewish (64.3%) control subjects ($P < 2.2 \times 10^{-16}$). These differences reflect the wide allelic variability reported in the literature, ranging from 83% (Ghanian) to 34% (Southwest Asians) (Ameyaw et al. 2001). Within European white populations, allele frequencies for the C3435 allele in the Portuguese, Spanish, German, and British have a range of 43%–52% (Ameyaw et al. 2001; Cascorbi et al. 2001; Bernal et al. 2003), consistent with the present findings of 45.4% in white non-Jewish control subjects. Therefore, the Jewish control C3435 allele frequency of 64.3% represents the highest reported frequency for white control individuals.

In contrast to a recent study of UC (Schwab et al. 2003), we found no association by either case-control or family-based association of the 3435T allele with UC, CD, or IBD. In that study, the allele frequencies for C3435 were reported for UC (43.3%), CD (53.2%), and control (51.7%) groups and concluded that the 3435T allele was associated with UC. However, all of these allele frequencies fall within the reported range for European cohorts; therefore, the observed differences might simply reflect subtle differences in population substructure. Furthermore, it is somewhat counterintuitive that a putative IBD risk allele (3435T) would have a lower allele frequency in Jewish compared with non-Jewish whites, given the higher disease prevalence of the former. In UC, the number of transmissions and the number of nontransmissions for the 3435T allele were essentially identical (49 to 50, respectively). Similarly, there was nonsignificant undertransmission of 3435T (as opposed to the more expected overtransmission) for both IBD (181 transmissions to 195 nontransmissions) and CD (232 transmissions to 247 nontransmissions).

Codon 893 of *MDR1* is located in the cytoplasmic loop occurring after the 10th transmembrane-spanning domain (Jones and George 1998). The Ala893 variant has a significantly lower transporter activity, compared with the 893Ser variant, as measured by both digoxin efflux in stable NIH-3T3 transfectants and plasma fexofenadine measurements in healthy volunteers (Kim et al. 2001). Therefore, the Ala893 IBD association is consistent with the *mdr1* knockout colitis model (Panwala et al. 1998). Given the marked undertransmission of the rare 893Thr variant, it will be of great interest to determine whether our observed protective association of this variant with IBD is secondary to higher transporter activity in NIH-3T3 transfectants, compared with the Ala893 variant. Because both serine and threonine residues are phosphorylated by a common group of kinases, it may be speculated that common mechanisms of phosphorylation of the 893Ser and 893Thr residues might account for increased transporter activity relative to the Ala893 variant, thereby increasing IBD risk. Whereas

regulation of activity has been reported to occur through phosphorylation of various residues of PGP (Chambers et al. 1993; Hardy et al. 1995; Germann et al. 1996), to our knowledge, no specific instances of phosphorylation of codon 893 have been reported; however, no groups have explicitly addressed possible allele-specific phosphorylation at this codon. A third possibility for explanation of the association of the Ala893 variant may be that this amino acid change alters specific substrate binding of potential toxins or other chemicals that may affect cellular stability or susceptibility to developing chronic unregulated inflammation. Such an alteration could be quite specific and unrelated to the transport activity of each variant with regard to the substrates, such as digoxin and fexofenadine, which have been tested in vitro.

The relationship of the Ala893Ser/Thr and C3435T polymorphisms to overall *MDR1* expression and activity is controversial. In general, *MDR1* transcript expression in the intestine and activity in human subjects has been more closely associated with the C3435T polymorphisms than with Ala893Ser/Thr polymorphisms. Using quantitative immunohistochemistry and western blot analysis, Hoffmeyer et al. (2000) evaluated intestinal PGP expression in intestinal biopsies from human volunteers. In addition, the plasma concentrations of orally administered digoxin, a PGP substrate, were recorded at specific time intervals to ascertain PGP activity. This study demonstrated increased intestinal expression and activity of PGP for C3435 homozygotes as compared with 3435T homozygotes. This study did not evaluate the Ala893Ser/Thr polymorphisms. Consistent with these findings, Johne et al. (2002) observed that 3435T-containing haplotypes had the greatest plasma digoxin concentrations, consistent with lowest PGP activity, regardless of the exon 21 genotype (Johne et al. 2002). In contrast, Nakamura et al. (2002) observed the opposite associations of mRNA expression levels in the duodenal enterocytes of Japanese subjects; that is, mRNA levels isolated from enterocytes of 3435T subjects were increased, compared with 3435C subjects (Nakamura et al. 2002). Population discrepancies between the *MDR1* expression studies, combined with the fact that the C3435T polymorphism is not within a known regulatory expression element, suggests that the C3435T polymorphism is in linkage disequilibrium with unknown regulatory polymorphism(s) directly influencing *MDR1* expression. Differing patterns of linkage disequilibrium in different populations could explain the different findings among these studies. In contrast, Kim et al. (2001), using the PGP substrate fexofenadine, observed that 3435T homozygotes and 893Ser homozygotes had increased net *MDR1* transport activity (as reflected by relatively lower fexofenadine plasma levels) compared with C3435 homozygotes or Ala893 homozygotes. These studies all assume a direct correlation between plasma drug levels and PGP activities. However, plasma levels likely reflect complex, unique pharmacokinetics of the absorption, distribution, and elimination for individual drugs. When the Ala893 and 893Ser variants were expressed in stable NIH-3T3 cells (isolating the effect of protein sequence from protein-expression levels and eliminating the complexity of drug pharmacokinetics), the Ala893 variant demonstrated lower transport activity (Kim et al. 2001). A model that may reconcile these diverse findings is as follows: the C3435T polymorphism is in linkage disequilibrium with a polymorphism that influences *MDR1* expression, whereas the Ala893Ser/Thr polymorphism influences innate PGP activity, perhaps in a substrate-specific or phosphorylation-dependent fashion.

Given the pharmacogenetic significance of *MDR1* combined with its importance in mediating intestinal epithelial homeostasis, as demonstrated in the *mdr1*-deficient mice (Panwala et al. 1998), additional studies to confirm a direct, causative role of the codon 893 variants in IBD should be performed. Replication in independent, large cohorts with IBD would be of primary importance. Because of the large size of the present cohort, highly significant association is observed, but the overall difference in association between the Ala893 and 893Ser variants, as measured by the ratios of transmission to nontransmission, is rather modest. Modest differences in common polymorphisms demonstrating slight functional effects probably represent the mainstay of risk alleles for complex, multigenic disorders. More marked association effects for the Ala893Ser/Thr polymorphism may eventually be observed, as additional risk alleles/susceptibility genes acting along the same pathogenetic pathway are identified. Such stratified analyses should not be performed randomly for all previously reported associations for a given disorder; rather, they should be grounded in a plausible model that is based on established mechanistic pathways. The marked nontransmission of the 893Thr variant is important, since it may be considered to be a highly significant, albeit rare, "protective" allele against developing IBD. Greater understanding of the endogenous substrates absorbed and/or excreted by intestinal epithelial cells harboring *MDR1* transporters may provide important insight into the pathogenesis and treatment of IBD.

Acknowledgments

This authors of this work are indebted to the patients with IBD and to their families, who gave of themselves to make this work possible. We thank Drs. Theodore M. Bayless, Stephen B. Hanauer, Mary Harris, Barbara Kirschner, and Themistocles Dassopoulos, who contributed data from families for this study; and Dr. Peter K. Gregersen, North Shore Long Island Jewish Research Institute, Manhasset, New York, who kindly provided

DNA samples from Ashkenazi Jewish control individuals. Mr. Franklin Nouvet and Ms. Yuqiong Wu contributed to the genotyping. This work was supported by the National Institutes of Health (NIH) (National Institute of Diabetes and Digestive and Kidney Diseases) grants R01 DK58189 and U01 DK062431 (to S.R.B.); RO1 DK55731, U01 DK62429, and U01 DK062422 (to J.H.C.); and R01 DK060867 (to R.H.D.); by NIH–General Clinical Research Center grants RR00052 (to S.R.B.) and RR00055 (to J.H.C.); by the Crohn's and Colitis Foundation of America (to S.R.B., J.H.C., and R.H.D.); by the Meyerhoff IBD Center (to S.R.B., C.H.P, D.M.R., L.W.D., G.R., and A.S.K.); by the Stewart W. Bainum family (to S.R.B.); by the Reva and David Logan Foundation and Gastrointestinal Research Foundation (to D.K.B, R.K., E.S., T.M., and J.H.C.); by the Johns Hopkins University Provost Undergraduate Research Award (to D.M.R.); by the American College of Gastroenterology Clinical Research Award (to J.P.A.); by The Scaife Family Foundation (to R.H.D.); and by the Israeli Society of Gastroenterology (support to A.S.K.).

Electronic-Database Information

URLs for data presented herein are as follows:

Genehunter, http://linkage.rockefeller.edu/soft/gh/ (for TDT and haplotype analysis [version 2.1])

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for CD, UC, and ABCB1/MDR1)

Pedigree Disequilibrium Test (PDT), http://wwwchg.mc.duke.edu/software/pdt.html (for conducting the PDT in general pedigrees)

Sib-Pair, http://www2.qimr.edu.au/davidD/sib-pair.html (for checking for Mendelian inconsistencies and Hardy-Weinberg equilibrium)

References

Ameyaw MM, Regateiro F, Li T, Liu X, Tariq M, Mobarek A, Thornton N, Folayan GO, Githang'a J, Indalo A, Ofori-Adjei D, Price-Evans DA, McLeod HL (2001) MDR1 pharmacogenetics: frequency of the C3435T mutation in exon 26 is significantly influenced by ethnicity. Pharmacogenetics 11: 217–221

Bernal ML, Sinues B, Fanlo A, Mayayo E (2003) Frequency distribution of C3435T mutation in exon 26 of the MDR1 gene in a Spanish population. Ther Drug Monit 25:107–111

Binder V (1998) Genetic epidemiology in inflammatory bowel disease. Dig Dis 16:351–355

Bonen DK, Ogura Y, Nicolae DL, Inohara N, Saab L, Tanabe T, Chen FF, Foster SJ, Duerr RH, Brant SR, Cho JH, Nunez G (2003) Crohn's disease-associated NOD2 variants share a signaling defect in response to lipopolysaccharide and peptidoglycan. Gastroenterology 124:140–146

Calado RT, Falcao RP, Garcia AB, Gabellini SM, Zago MA, Franco RF (2002) Influence of functional MDR1 gene polymorphisms on P-glycoprotein activity in CD34+ hematopoietic stem cells. Haematologica 87:564–568

Cascorbi I, Gerloff T, Johne A, Meisel C, Hoffmeyer S, Schwab M, Schaeffeler E, Eichelbaum M, Brinkmann U, Roots I (2001) Frequency of single nucleotide polymorphisms in the P-glycoprotein drug transporter MDR1 gene in white subjects. Clin Pharmacol Ther 69:169–174

Chamaillard M, Philpott D, Girardin SE, Zouali H, Lesage S, Chareyre F, Bui TH, Giovannini M, Zaehringer U, Penard-Lacronique V, Sansonetti PJ, Hugot JP, Thomas G (2003) Gene-environment interaction modulated by allelic heterogeneity in inflammatory diseases. Proc Natl Acad Sci USA 100:3455–3460

Chambers TC, Pohl J, Raynor RL, Kuo JF (1993) Identification of specific sites in human P-glycoprotein phosphorylated by protein kinase C. J Biol Chem 268:4592–4595

Cho JH, Nicolae DL, Gold LH, Fields CT, LaBuda MC, Rohal PM, Pickles MR, Qin L, Fu Y, Mann JS, Kirschner BS, Jabs EW, Weber J, Hanauer SB, Bayless TM, Brant SR (1998) Identification of novel susceptibility loci for inflammatory bowel disease on chromosomes 1p, 3q, and 4q: evidence for epistasis between 1p and IBD1. Proc Natl Acad Sci USA 95: 7502–7507

Choi KH, Chen CJ, Kriegler M, Roninson IB (1988) An altered pattern of cross-resistance in multidrug-resistant human cells results from spontaneous mutations in the mdr1 (P-glycoprotein) gene. Cell 53:519–529

Duerr RH, Barmada MM, Zhang L, Pfützer R, Weeks DE (2000) High-density genome scan in Crohn disease shows confirmed linkage to chromosome 14q11-12. Am J Hum Genet 66: 1857–1862

Duffy DL (1997) Sib-pair: a program for non-parametric linkage/association analysis. Am J Hum Genet 61:A197

Farrell RJ, Murphy A, Long A, Donnelly S, Cherikuri A, O'Toole D, Mahmud N, Keeling PW, Weir DG, Kelleher D (2000) High multidrug resistance (P-glycoprotein 170) expression in inflammatory bowel disease patients who fail medical therapy. Gastroenterology 118:279–288

Furuno T, Landi MT, Ceroni M, Caporaso N, Bernucci I, Nappi G, Martignoni E, Schaeffeler E, Eichelbaum M, Schwab M, Zanger UM (2002) Expression polymorphism of the blood-brain barrier component P-glycoprotein (MDR1) in relation to Parkinson's disease. Pharmacogenetics 12:529–534

Germann UA, Chambers TC, Ambudkar SV, Licht T, Cardarelli CO, Pastan I, Gottesman MM (1996) Characterization of phosphorylation-defective mutants of human P-glycoprotein expressed in mammalian cells. J Biol Chem 271:1708–1716

Girardin SE, Boneca IG, Viala J, Chamaillard M, Labigne A, Thomas G, Philpott DJ, Sansonetti PJ (2003) Nod2 is a general sensor of peptidoglycan through muramyl dipeptide (MDP) detection. J Biol Chem 278:8869–8872

Hampe J, Schreiber S, Shaw SH, Lau KF, Bridger S, Macpherson AJS, Cardon LR, Sakul H, Harris TJR, Buckler A, Hall J, Stokkers P, van Deventer SJJ, Nürnberg P, Mirza MM, Lee JCW, Lennard-Jones JE, Mathew CG, Curran ME (1999) A genomewide analysis provides evidence for novel linkages in inflammatory bowel disease in a large European cohort. Am J Hum Genet 64:808–816

Hardy SP, Goodfellow HR, Valverde MA, Gill DR, Sepulveda V, Higgins CF (1995) Protein kinase C-mediated phosphorylation of the human multidrug resistance P-glycoprotein regu-

lates cell volume-activated chloride channels. Embo J 14:68–75

Higgins CF, Gottesman MM (1992) Is the multidrug transporter a flippase? Trends Biochem Sci 17:18–21

Hitzl M, Drescher S, van der Kuip H, Schaffeler E, Fischer J, Schwab M, Eichelbaum M, Fromm MF (2001) The C3435T mutation in the human MDR1 gene is associated with altered efflux of the P-glycoprotein substrate rhodamine 123 from CD56+ natural killer cells. Pharmacogenetics 11:293–298

Hoffmeyer S, Burk O, von Richter O, Arnold HP, Brockmoller J, Johne A, Cascorbi I, Gerloff T, Roots I, Eichelbaum M, Brinkmann U (2000) Functional polymorphisms of the human multidrug-resistance gene: multiple sequence variations and correlation of one allele with P-glycoprotein expression and activity in vivo. Proc Natl Acad Sci USA 97:3473–3478

Hugot JP, Chamaillard M, Zouali H, Lesage S, Cezard JP, Belaiche J, Almer S, Tysk C, O'Morain CA, Gassull M, Binder V, Finkel Y, Cortot A, Modigliani R, Laurent-Puig P, Gower-Rousseau C, Macry J, Colombel JF, Sahbatou M, Thomas G (2001) Association of NOD2 leucine-rich repeat variants with susceptibility to Crohn's disease. Nature 411: 599–603

Hugot JP, Laurent-Puig P, Gower-Rousseau C, Olson JM, Lee JC, Beaugerie L, Naom I, Dupas JL, Van Gossum A, Orholm M, Bonaiti-Pellie C, Weissenbach J, Mathew CG, Lennard-Jones JE, Cortot A, Colombel JF, Thomas G (1996) Mapping of a susceptibility locus for Crohn's disease on chromosome 16. Nature 379:821–823

Inohara N, Ogura Y, Fontalba A, Gutierrez O, Pons F, Crespo J, Fukase K, Inamura S, Kusumoto S, Hashimoto M, Foster SJ, Moran AP, Fernandez-Luna JL, Nunez G (2003) Host recognition of bacterial muramyl dipeptide mediated through NOD2: implications for Crohn's disease. J Biol Chem 278: 5509–5512

Johne A, Kopke K, Gerloff T, Mai I, Rietbrock S, Meisel C, Hoffmeyer S, Kerb R, Fromm MF, Brinkmann U, Eichelbaum M, Brockmoller J, Cascorbi I, Roots I (2002) Modulation of steady-state kinetics of digoxin by haplotypes of the P-glycoprotein MDR1 gene. Clin Pharmacol Ther 72:584–594

Jones PM, George AM (1998) A new structural model for P-glycoprotein. J Membr Biol 166:133–147

Kim RB, Leake BF, Choo EF, Dresser GK, Kubba SV, Schwarz UI, Taylor A, Xie HG, McKinsey J, Zhou S, Lan LB, Schuetz JD, Schuetz EG, Wilkinson GR (2001) Identification of functionally variant MDR1 alleles among European Americans and African Americans. Clin Pharmacol Ther 70:189–199

Kong A, Cox NJ (1997) Allele-sharing models: LOD scores and accurate linkage tests. Am J Hum Genet 61:1179–1188

Kruglyak L, Daly MJ, Reeve-Daly MP, Lander ES (1996) Parametric and nonparametric linkage analysis: a unified multipoint approach. Am J Hum Genet 58:1347–1363

Kruglyak L, Nickerson DA (2001) Variation is the spice of life. Nat Genet 27:234–236

Lown KS, Mayo RR, Leichtman AB, Hsiao HL, Turgeon DK, Schmiedlin-Ren P, Brown MB, Guo W, Rossi SJ, Benet LZ, Watkins PB (1997) Role of intestinal P-glycoprotein (mdr1) in interpatient variation in the oral bioavailability of cyclosporine. Clin Pharmacol Ther 62:248–260

Ma Y, Ohmen JD, Li Z, Bentley LG, McElree C, Pressman S, Targan SR, Fischel-Ghodsian N, Rotter JI, Yang H (1999) A genome-wide search identifies potential new susceptibility loci for Crohn's disease. Inflamm Bowel Dis 5:271–278

Markianos K, Daly MJ, Kruglyak L (2001) Efficient multipoint linkage analysis through reduction of inheritance space. Am J Hum Genet 68:963–977

Martin ER, Monks SA, Warren LL, Kaplan NL (2000) A test for linkage and association in general pedigrees: the pedigree disequilibrium test. Am J Hum Genet 67:146–154

Nakamura T, Sakaeda T, Horinouchi M, Tamura T, Aoyama N, Shirakawa T, Matsuo M, Kasuga M, Okumura K (2002) Effect of the mutation (C3435T) at exon 26 of the MDR1 gene on expression level of MDR1 messenger ribonucleic acid in duodenal enterocytes of healthy Japanese subjects. Clin Pharmacol Ther 71:297–303

Ogura Y, Bonen DK, Inohara N, Nicolae DL, Chen FF, Ramos R, Britton H, Moran T, Karaliuskas R, Duerr RH, Achkar JP, Brant SR, Bayless TM, Kirschner BS, Hanauer SB, Nunez G, Cho JH (2001) A frameshift mutation in NOD2 associated with susceptibility to Crohn's disease. Nature 411:603–606

Orholm M, Munkholm P, Langholz E, Nielsen OH, Sorensen IA, Binder V (1991) Familial occurrence of inflammatory bowel disease. N Engl J Med 324:84–88

Paavola-Sakki P, Ollikainen V, Helio T, Halme L, Turunen U, Lahermo P, Lappalainen M, Farkkila M, Kontula K (2003) Genome-wide search in Finnish families with inflammatory bowel disease provides evidence for novel susceptibility loci. Eur J Hum Genet 11:112–120

Panwala CM, Jones JC, Viney JL (1998) A novel model of inflammatory bowel disease: mice deficient for the multiple drug resistance gene, mdr1a, spontaneously develop colitis. J Immunol 161:5733–5744

Peeters M, Nevens H, Baert F, Hiele M, de Meyer AM, Vlietinck R, Rutgeerts P (1996) Familial aggregation in Crohn's disease: increased age-adjusted risk and concordance in clinical characteristics. Gastroenterology 111:597–603

Podolsky DK (2002) Inflammatory bowel disease. N Engl J Med 347:417–429

Rioux JD, Silverberg MS, Daly MJ, Steinhart AH, McLeod RS, Griffiths AM, Green T, Brettin TS, Stone V, Bull SB, Bitton A, Williams CN, Greenberg GR, Cohen Z, Lander ES, Hudson TJ, Siminovitch KA (2000) Genomewide search in Canadian families with inflammatory bowel disease reveals two novel susceptibility loci. Am J Hum Genet 66:1863–1870

Satsangi J, Parkes M, Louis E, Hashimoto L, Kato N, Welsh K, Terwilliger JD, Lathrop GM, Bell JI, Jewell DP (1996) Two stage genome-wide search in inflammatory bowel disease provides evidence for susceptibility loci on chromosomes 3, 7 and 12. Nat Genet 14:199–202

Schinkel AH (1997) The physiological function of drug-transporting P-glycoproteins. Semin Cancer Biol 8:161–170

Schinkel AH, Mayer U, Wagenaar E, Mol CA, van Deemter L, Smit JJ, van der Valk MA, Voordouw AC, Spits H, van Tellingen O, Zijlmans JM, Fibbe WE, Borst P (1997) Normal viability and altered pharmacokinetics in mice lacking mdr1-type (drug-transporting) P-glycoproteins. Proc Natl Acad Sci USA 94:4028–4033

Schinkel AH, Wagenaar E, Mol CA, van Deemter L (1996) P-

glycoprotein in the blood-brain barrier of mice influences the brain penetration and pharmacological activity of many drugs. J Clin Invest 97:2517–2524

Schinkel AH, Wagenaar E, van Deemter L, Mol CA, Borst P (1995) Absence of the mdr1a P-Glycoprotein in mice affects tissue distribution and pharmacokinetics of dexamethasone, digoxin, and cyclosporin A. J Clin Invest 96:1698–1705

Schwab M, Schaeffeler E, Marx C, Fromm MF, Kaskas B, Metzler J, Stange E, Herfarth H, Schoelmerich J, Gregor M, Walker S, Cascorbi I, Roots I, Brinkmann U, Zanger UM, Eichelbaum M (2003) Association between the C3435T MDR1 gene polymorphism and susceptibility for ulcerative colitis. Gastroenterology 124:26–33

Siddiqui A, Kerb R, Weale ME, Brinkmann U, Smith A, Goldstein DB, Wood NW, Sisodiya SM (2003) Association of multidrug resistance in epilepsy with a polymorphism in the drug-transporter gene ABCB1. N Engl J Med 348:1442–1448

Siegmund W, Ludwig K, Giessmann T, Dazert P, Schroeder E, Sperker B, Warzok R, Kroemer HK, Cascorbi I (2002) The effects of the human MDR1 genotype on the expression of duodenal P-glycoprotein and disposition of the probe drug talinolol. Clin Pharmacol Ther 72:572–583

Sparreboom A, van Asperen J, Mayer U, Schinkel AH, Smit JW, Meijer DK, Borst P, Nooijen WJ, Beijnen JH, van Tellingen O (1997) Limited oral bioavailability and active epithelial excretion of paclitaxel (Taxol) caused by P-glycoprotein in the intestine. Proc Natl Acad Sci USA 94:2031–2035

Subhani JMS, Ounder RE, Wakefield AJ (1998) Concordance rates of twins and siblings in inflammatory bowel disease. Gut Suppl 42:A40

Thompson NP, Driscoll R, Pounder RE, Wakefield AJ (1996) Genetics versus environment in inflammatory bowel disease: results of a British twin study. BMJ 312:95–96

Tysk C, Lindberg E, Jarnerot G, Floderus-Myrhed B (1988) Ulcerative colitis and Crohn's disease in an unselected population of monozygotic and dizygotic twins: a study of heritability and the influence of smoking. Gut 29:990–996

Ueda K, Cardarelli C, Gottesman MM, Pastan I (1987) Expression of a full-length cDNA for the human "MDR1" gene confers resistance to colchicine, doxorubicin, and vinblastine. Proc Natl Acad Sci USA 84:3004–3008

van Helvoort A, Smith AJ, Sprong H, Fritzsche I, Schinkel AH, Borst P, van Meer G (1996) MDR1 P-glycoprotein is a lipid translocase of broad specificity, while MDR3 P-glycoprotein specifically translocates phosphatidylcholine. Cell 87:507–517

Verstuyft C, Schwab M, Schaeffeler E, Kerb R, Brinkmann U, Jaillon P, Funck-Brentano C, Becquemont L (2003) Digoxin pharmacokinetics and MDR1 genetic polymorphisms. Eur J Clin Pharmacol 58:809–812

Weir DG (1996) Genetic-data analysis II: methods for discrete population genetic data. Sinauer, Sunderland, MA

Williams CN, Kocher K, Lander ES, Daly MJ, Rioux JD (2002) Using a genome-wide scan and meta-analysis to identify a novel IBD locus and confirm previously identified IBD loci. Inflamm Bowel Dis 8:375–381

Yamauchi A, Ieiri I, Kataoka Y, Tanabe M, Nishizaki T, Oishi R, Higuchi S, Otsubo K, Sugimachi K (2002) Neurotoxicity induced by tacrolimus after liver transplantation: relation to genetic polymorphisms of the ABCB1 (MDR1) gene. Transplantation 74:571–572